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Effect of humic acid on the growth of seedling tomato (*Solanum lycopersicum*) and melon *Cucumis melo*)

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ABSTRACT
This study evaluated the effect of HS extracted from mineral coal on the growth of tomato (Solanum lycopersicum) and melon (Cucumis melo) plants to determine the role that humic substances (HS) play as promoters of plant growth. Three concentrations of HS were evaluated in 200 grams of soil. The fertilizer (urea 0.3%) and humic acid in concentrations of 0.05%, 0.1%, and 0.2% were added directly and at the same time to the amount of soil, using a control sample without the addition of humic acid. Increase measurements were made at three-day intervals up to 45 days. Plants treated with high concentrations of HS demonstrated a significant increase in roots ($p>0.05$). In both plants, the concentration of 0.2% in HS showed the greatest increase in growth, with the melon plant showing the greatest variation as time passed, with its highest peak in 36 days (13.1 ± 0.05cm) of the experiment, while in the tomato plant the maximum growth occurred in 30 days (9.2 ± 0.01 cm). The Dunnett test showed that there was no statistically significant difference between the control and the concentrations of 0.05% and 0.1% ($p>0.05$), while among the control and the soil sample with a concentration of 0.2% there was a statistically significant difference ($p<0.05$). The results show a notable influence of humic acid on the growth of the studied plants, especially at high concentrations.

Keywords: fertilizer, horticulture, humic acid, soils.

Efeito do ácido húmico no crescimento de mudas de tomate (*Solanum lycopersicum*) e melão (*Cucumis melo*)

RESUMO
Para determinar o papel que as substâncias húmicas (HS) desempenham como promotores do crescimento vegetal, foi avaliado o efeito do HS extraído do carvão mineral no crescimento de plantas de tomate (Solanum lycopersicum) e melão (Cucumis melo). Três concentrações de HS foram avaliadas em 200 gramas de solo. O fertilizante (uréia 0,3%) e ácido húmico nas concentrações de 0,05%, 0,1% e 0,2% foram adicionados diretamente e ao mesmo tempo na quantidade de solo, utilizando uma amostra controle sem adição de ácido húmico. As medições de aumento foram feitas em intervalos de três dias até 45 dias. Plantas tratadas com altas concentrações de HS demonstraram um aumento significativo nas raízes ($p> 0,05$). Em ambas

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as plantas, a concentração de 0,2% no HS apresentou o maior aumento de crescimento, com o melão apresentando a maior variação com o passar do tempo, com seu maior pico nos 36 dias (13,1 ± 0,05cm) do experimento, enquanto no no tomateiro o máximo crescimento ocorreu em 30 dias (9,2 ± 0,01 cm). O teste de Dunnett mostrou que não houve diferença estatisticamente significativa entre o controle e as concentrações de 0,05% e 0,1% (p> 0,05), enquanto entre o controle e a amostra de solo com concentração de 0,2% houve diferença estatisticamente significativa (p <0,05). Os resultados obtidos mostram uma notável influência do ácido húmico no crescimento das plantas estudadas, principalmente em altas concentrações.

Palavras-chave: ácido húmico, fertilizante, horticultura, solos.

1. INTRODUCTION

Humification is the chemical and microbiological process that transforms the dead remains into humic substances. It is the second-most extensive process on earth after photosynthesis, involving from 20 to 75 gigatons of carbon each year (Bacilio *et al.*, 2017; Marschner *et al.*, 2008). Humic substances are found in soils, sediments and natural waters like rivers, lakes and oceans (Wu *et al.*, 2017)

They also represent a large proportion of the organic matter in peat swamps, carbonaceous shales, dark coals (lignites), and sewage. Humic materials contribute to the yellow and brown colors of leaf litter and composts, the dark brown or black colors of topsoil, and when in high concentrations, the brown stain in freshwater creeks and lakes (Khorasaninejad *et al.*, 2018). They have even been detected on the Antarctic continent. The understanding of humic substances and the way they work are both substantially more sophisticated than the basic knowledge of fertilizers. Generally, humic substances are considered as a series of relatively high-molecular-weight, brown to black colored substances formed by secondary synthesis reactions. The term is employed as a generic name to describe the colored material or its fractions obtained based on solubility characteristics (Kipton *et al.*, 1992).

The fraction called humic acid is not soluble in water under acidic conditions (pH<2) but is soluble at higher pH values (Morozesk *et al.*, 2017). Many experts currently believe that all dark-colored humic substances are part of a system of closely related but not completely identical, high-molecular-weight polymers. According to this concept, differences between humic and fulvic acids can be explained by variations in molecular weight, the number of functional groups (carboxyl, phenolic OH), and the extent of polymerization. Among the humic substances are the humic acids, which are formed by nuclei of aromatic and polyaromatic compounds. Humic acids have different functional groups in their structure that give them the capacity to exercise various functions in the soil-plant relationship (Pantoja *et al.*, 2016; Canellas *et al.*, 2010).

These substances are formed by the decomposition of plant and animal material deposited in the soil (Wu *et al.*, 2017; Maji *et al.*, 2017). The organic matter is initially degraded, then depolymerized, and finally by the action of microorganisms produces new components with a high degree of dark-colored polymerization (Canellas and Olivares, 2014). Humic substances (HS) can cause changes in the root and architecture and can increase dynamics, which result in a larger root size, better branching, and/or a higher density of root hair with a greater surface area, for this reason, they have been widely recognized as promoters of plant growth (Brown *et al.*, 2013).

The study of Trevis *et al.* (2010) found that humic substances (SH) stimulate the growth of the stem, root, leaves, fresh, and dry mass, size, and quality of the fruits; as well as crop yields. In support of this, a previous study showed that the dry mass of herbaceous plant shoots and roots increased by about 22% in response to the exogenous application of humic and fulvic acid (Rose *et al.*, 2014). Humic compounds such as humic acid and fulvic acid have been shown to stimulate plant growth in terms of increasing plant height and dry or fresh weight as well as enhancing nutrient uptake (Felle, 2002). These effects seem to depend on the concentration and source of the substance and the plant species (Quaggiotti *et al.*, 2004).

Humic substances are classified depending on the separation process used; among the major components of humus are acids soluble in the acid medium, known as fulvic acids (AF), and insoluble in acid medium, called humic acids (AH) (Canellas *et al.*, 2015). In many studies, humic and fulvic acid preparations were reported to increase the uptake of mineral elements. Due to the positive effect of humic substances on the visible growth of plants, these chemicals have been widely used by growers instead of other substances such as pesticides, etc. This, however, has led to growers using higher amounts of these substances. The carboxylic, phenolic, carbonyl, methoxide, and aliphatic groups present in the structure of the humic substances make this a highly complex structural matrix, which has uniformity in each of its units, conformed by condensed aromatic rings containing carboxylic groups (Gomes De Melo *et al.*, 2016).

It is estimated that approximately a quarter of the molecular weight of the humic substances is due to the oxygenated groups, mainly carboxylic groups that increase with the degree of humification of the organic matter and that can form carboxylates with metals present in the medium, phenolic groups that they form in the initial stages of humification and carbonyls groups that by oxidation reactions form carboxylic groups (Fischer, 2017; Gomes De Melo *et al.*, 2016; Cantero *et al.*, 2015; Pédrot and Mélanie, 2010). Humic substances play a very important role in nature; this is due to their present oxygenated functional groups, and they can participate in cation retention processes that are essential for plants as well as retaining heat on the surface due to their dark color (Pédrot and Mélanie, 2010). These substances can be used as fertilizers, given their ability to retain metals useful for agriculture; they can also be used in the removal of toxic substances in aqueous effluents (Nardi *et al.*, 2016; Sun *et al.*, 2015; Tang *et al.*, 2014; Kalina *et al.*, 2013).

A significant source of humic substances in the low range carbons, which have a high content of oxygenated groups and a part of their structure quite similar to that of humic acids (Motta and Santana, 2013). This allows being able to perform the extraction with alkaline solutions of the humic material of this type of coal. Moderate oxidation reactions in mineral carbons may increase the content of humic substances in their structure, which also leads to higher percentages of extraction of this type of materials (Zhiyuan *et al.*, 2012).

This research assessed the effect of humic acid obtained from low-grade coal from the Montelíbano mine (Córdoba-Colombia) on tomato and melon plants grown in low-fertility soils.

2. MATERIAL AND METHODS

2.1. Mineral material

In this research, low-grade coal from the mine located in the rural area of Montelibano (7°58'45.0" N -75°25'12.7" W) in the department of Córdoba-Colombia is used as the starting mineral material. All the experiments were carried out at the facilities of the Faculty of Engineering, Food Engineering Program of the University of Cartagena.

2.2. Preparation of the carbon sample

The sample was first crushed and sieved to a particle size between 3.1 and 7.1 mm through a sieve with a mesh number of 6. A portion of 100 g of the material was demineralized for 1 hour at room temperature with hydrochloric acid 0.5 M. Before the oxidation process, the mineral starting sample was subjected to a debitumization process using an ethanol-benzene mixture (1:1 v/v) at reflux for 24 hours. The solvent was removed by distillation in Soxhlet and vacuum filtration with distilled water. The remaining moisture was removed by drying at 80°C for 12 hours (Anillo *et al.*, 2013). Subsequently, oxidation was carried out in an aqueous medium with 30% hydrogen peroxide and concentrated acetic acid. This system was heated to a temperature of 60°C with continuous agitation for a space of 12 hours (Anillo *et al.*, 2013).

2.3. Production of the humic acid

A volume of 100 mL of 0.1 M NaOH was added to 5 g of oxidized carbon; this system was maintained at 60°C for 1 h in continuous agitation. The resulting solution was filtered under vacuum and then 100 mL of 0.1 M HCl was added. This system was kept at rest for 24h and was subsequently centrifuged at 3600 rpm for 10 minutes. The colloid obtained after the centrifugation was washed with ethanol and heated to a temperature of 100°C for one hour and subsequently analyzed. The percentage of humic acids extracted was quantified to the mass of extracted acids obtained from the initial weight of the oxidized carbon samples (Anillo *et al.*, 2013).

2.4. Soil strengthening

The soils were fortified with humic acid and urea. Two hundred g of soil was added directly to fertilizer and humic acid at a concentration of 0.05%, 0.1%, and 0.2% directly concerning the amount of soil, using a sample control, containing urea (0.3%) as fertilizer, without the addition of humic acid. The amount of irrigation water for the tomato seedlings was 1 to 1.5 L/day by sprinkling and for the melon it was 3 liters with 4 irrigations per week, which was done by drip irrigation, taking into account a good distribution of irrigation throughout the crop cycle, avoiding subjecting the crop to deficiencies or excess of water. The soils were enriched with nitrogen, phosphorus, potassium, magnesium and calcium.

2.5. Agronomic management

The tomato and melon seeds were supplied by farmers in the northern part of the department of Bolivar, Colombia. They were grayish in color, flattened oval in shape, and ranged in size from 2-6 mm in diameter and 2 mm in length. In the case of melon, these seeds were white to brown to orange-yellowish, smooth morphology with some flattening, elongated, small and pointed at one end, with a dimension of ¼ to ¾ inch long.

The seeds were sown uniformly in an open space. The temperature during the seedling growth period was between 26 (minimum) and 31°C (maximum), with an average relative humidity of 80%. The soil pH range for tomato seedling development was 5.9 to 6.5 and for melon seedlings between 6 and 7.

2.6. Experimental design

In this research, a unifactorial experimental design was used, complemented at random with three repetitions, where the tomato (*Solanum lycopersicum*) plants and melon (*Cucumis melo*) seeds were planted in the strengthened soils and the control. After the first day of planting, a growth measurement was made at a time interval of 3 days to complete a total of 45 days. All soil samples were hydrated by irrigation.

2.7. Statistical analysi

The results were statistically analyzed, taking as a response variable the measure of plant growth. The analyses included one-way ANOVA (unidirectional), using measurements of the growth of the plants to determine statistically significant differences ($P<0.05$) between the samples. The multiple comparison tests of Dunnett were used with the purpose of making comparisons of plant growth in the presence of humic acid and the control, in this case, urea, at a confidence level of 95%.

3. RESULTS AND DISCUSSION

The growth of tomato and melon plants was significantly influenced by each of the different concentrations of humic acids in the fertilizer medium. The tomato plant presented a similar behavior in a study carried out by Rodríguez (2019, reported by Cantero *et al.*, 2015). Various studies demonstrate the importance of the use of bioactive substances. For example, humic substances can directly affect the metabolism of plants by exerting an influence on the transport of ions, facilitating their absorption, increasing respiration and the speed of enzymatic reactions. This leads to greater energy production, increases the chlorophyll content, increases the synthesis of nucleic acids, the selective effect on protein synthesis and the increase or inhibition of various enzymes, resulting in a positive effect on plant growth (Reyes-Pérez *et al.*, 2020; Shah *et al.*, 2018; You *et al.*, 2018).

These structures are capable of chelating metals, being able to influence the physico-chemical structure of the protoplasm of plants, thereby increasing the permeability of plant membranes, which increases plants' assimilation of nitrogen, phosphorus, potassium and other microelements present in the soil.

3.1. Tomato plant growth behavior

Current evidence suggests that the biostimulant effects of humic substances are characterized by both structural and physiological changes in roots and shoots related to absorption, assimilation and distribution of nutrients (efficiency traits in the use of nutrients). In addition, they can induce changes in the primary and secondary metabolism in the tomato plant related to tolerance to abiotic stress that participates in the modulation of plant growth (Canellas *et al.*, 2015).

Figure 1 shows the growth of the tomato plants (*Solanum lycopersicum*) versus the concentration of humic acid in the soil, with the concentration of 0.2% showing a considerable increase in the growth of the plant compared to the control and the other two concentrations (average growth of 6.88 ± 2.99 cm).

The analysis of ANOVA shows that at the concentration of 0.2% the statistically significant difference with the control is evidenced ($p<0.05$), while at the concentrations of 0.05% and 0.1% with the control there is no significant difference ($p>0.05$).

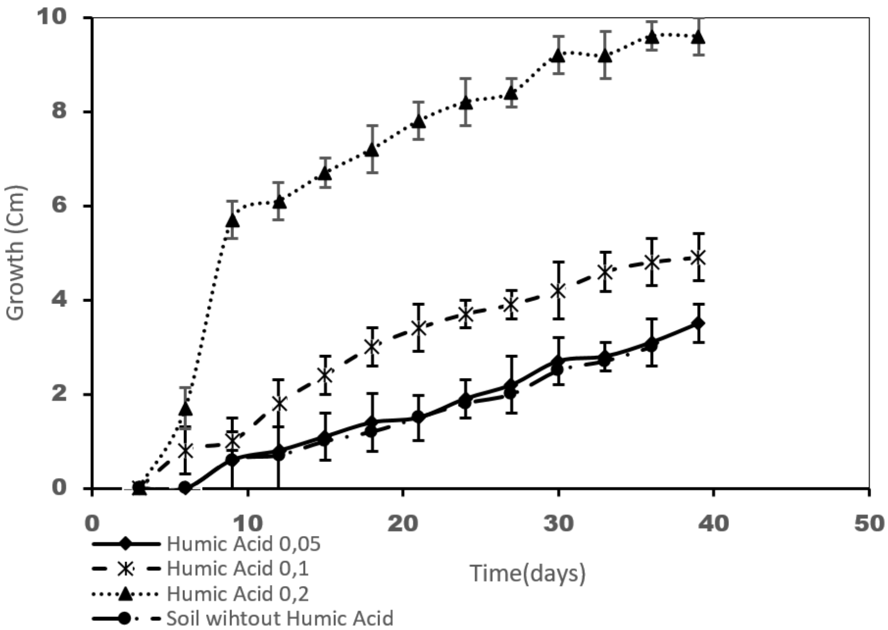

Figure 1. Growth rate of *Solanum lycopersicum* (tomato) using different concentrations of humic acid.

This growth can be noticed as the days passed, with an increase in plant growth appearing on Day 9 (Table 1). Figure 3a shows the growth of the plant at the 0.05% concentration is very similar to the control plants (mean growth of 1.66 ± 1.15 cm and 1.41 ± 1.01cm, respectively); while the concentration of 0.1% at 12 days begins a significant growth compared to the control at the concentration of 0.05%. However, as shown in Figure 3a, at the humic acid concentration of 0.2%, abrupt growth of the plant occurs 9 days after sowing, causing a large, statistically significant difference between the control and the other concentrations (Colpas *et al.*, 2018).

According to the results, the tomato plant presents growth dependent on the concentration of humic acid, reaching a maximum peak (9.20 ± 0.01 cm) on Day 30 and then attaining a steady state.

Table 1. Tomatoes (*Solanum lycopersicum*) grown in soils fertilized with urea and different concentrations of humic acid.

Days of growth	Soils fertilized with urea and fortified with humic acid (%)			
	0.05%	0.1%	0.2%	Control
3	-	-	-	-
6	-	0.8 ± 0.02[a]	1.7 ± 0.04[c]	-
9	0.6 ± 0.019[a]	1.0 ± 0.02[b]	5.7 ± 0.02[c]	0.6 ± 0.06[a]
12	0.8 ± 0.019[a]	1.8 ± 0.03[b]	6.1 ± 0.08[c]	0.7 ± 0.02[a]
15	1.1 ± 0.015[a]	2.4 ± 0.01[b]	6.7 ± 0.21[c]	1.0 ± 0.01[a]
18	1.4 ± 0.022[a]	3.0 ± 0.02[b]	7.2 ± 0.01[c]	1.2 ± 0.02[b]
21	1.5 ± 0.018[a]	3.4 ± 0.01[b]	7.8 ± 0.03[c]	1.5 ± 0.05[a]
24	1.9 ± 0.030[a]	3.7 ± 0.03[b]	8.2 ± 0.02[c]	1.8 ± 0.03[a]
27	2.2 ± 0.023[a]	3.9 ± 0.05[b]	8.4 ± 0.02[c]	2.0 ± 0.03[d]
30	2.7 ± 0.005[a]	4.2 ± 0.03[b]	9.2 ± 0.01[c]	2.5 ± 0.01[d]
33	2.8 ± 0.027[a]	4.6 ± 0.11[b]	9.2 ± 0.03[c]	2.7 ± 0.01[a]
36	3.1 ± 0.009[a]	4.8 ± 0.01[b]	9.6 ± 0.01[c]	3.0 ± 0.01[a]
39	3.5 ± 0.002[a]	4.9 ± 0.01[a]	9.6 ± 0.03[c]	-
42	-	-	-	-
45	-	-	-	-

Different letters in the same row differ significantly ($p < 0.05$). The values represent the mean ± S.E. ($n = 3$). The growth of the plant is expressed in centimeters (Cm). The - denotes absence of growth.

3.2. Melon plant growth behavior

Initial concentrations of 0.05 and 0.1% are similar to the control only at 18 days (Figure 2), corroborated with the Dunnett multiple comparison test, where no statistically significant differences are found between these concentrations and the control (Figure 3b). At a concentration of 0.2 as shown, there are differences in the control. It is interesting to note that the growth of the melon plant at a concentration of 0.2% increases as time goes on, observing an almost exponential growth starting on the ninth day, reaching a maximum length on Day 36 (average of 8.04 ± 4.08 cm), where a slight steady state is noticeable (Table 2).

The variance analysis shows that there is a statistically significant difference ($p<0.05$) as the concentration of humic acid increases. Thus, favoring the growth of the melon. The Dunnett test shows that there is a statistically significant difference between the control and the 0.2% concentration. It can be highlighted that the growth of the melon plant is favored when the humic acid concentrations increase in the soil. While at low concentrations, this growth is similar when this plant grows in soil without humic acid. The results of the humic acid (HS) application test show that all treatments cause significant increases in the total surface area of roots. The increase in leaf area and total root surface caused by treatments with different types of humic acid contributes to the hypothesis of a biostimulant action in plant development (Nebbioso and Piccolo. 2012). Numerous studies have been conducted on the effects of humic substances and commercial humic products on the growth and yield of plants. The reviewed articles demonstrated a positive response to germination and seedling growth, root initiation and shoot growth and development to a variety of humic extracts from a variety of sources.

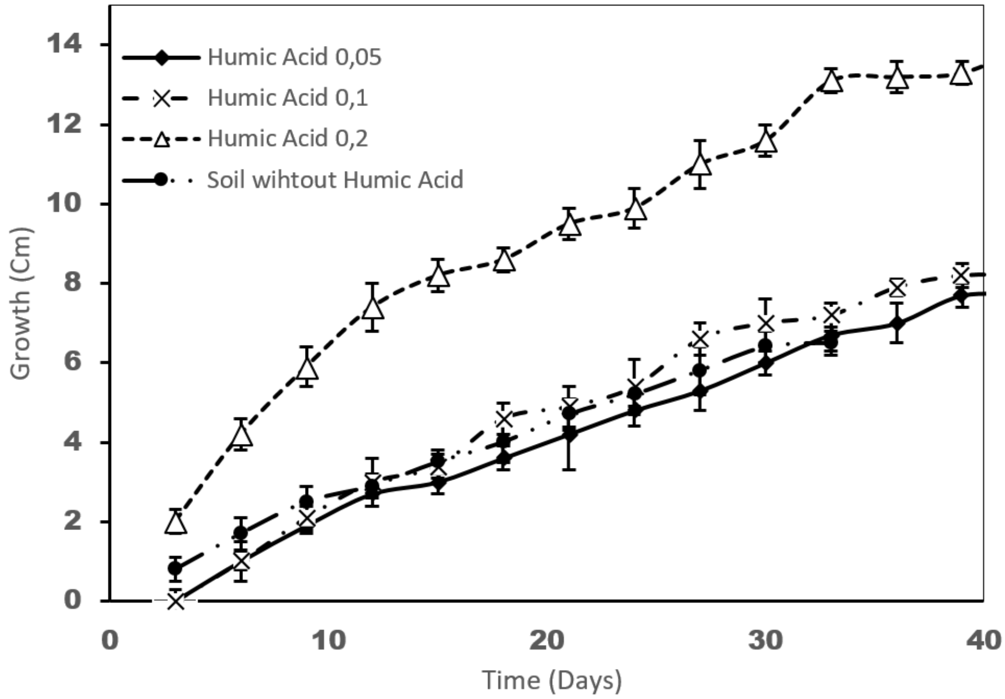

Figure 2. Growth rate of *Cucumis melo* (melon) using different concentrations of humic acid.

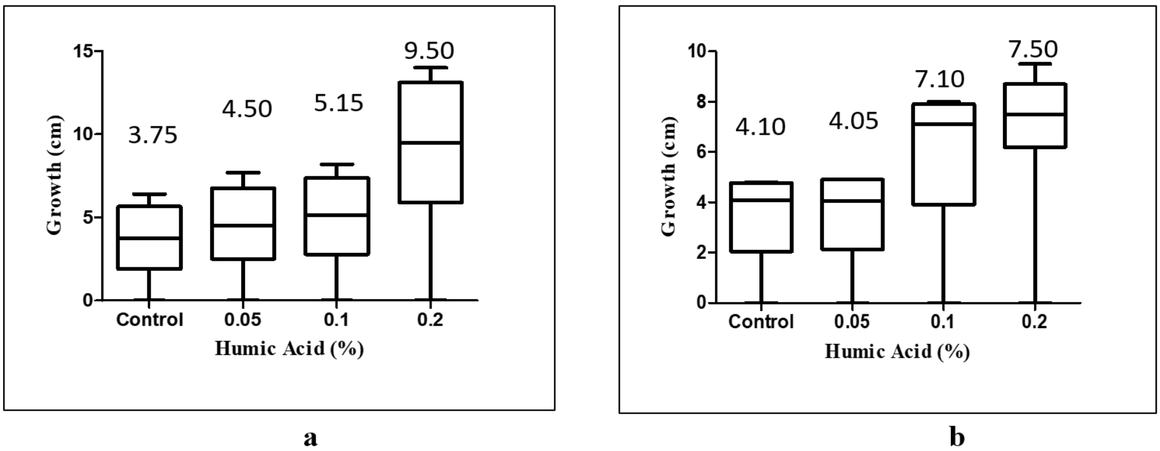

Figure 3. Growth variations in the presence of different concentrations of humic acids compared to the control (without humic acid). a) *Solanum lycopersicum* (tomato) and b) *Cucumis melo* (melon).

Table 2. Melon plant growth (*Cucumis melo*) cultivated in soils fertilized with urea and different concentrations of humic acid.

Days of growth	Soils fertilized with urea and fortified with humic acid (%)			
	0.05%	0.1%	0.2%	Control
3	-	-	-	-
6	1.0 ± 0.01[a]	1.0 ± 0.03[b]	2.0 ± 0.03[c]	0.8 ± 0.01[d]
9	1.9 ± 0.05[a]	2.1 ± 0.02[b]	4.2 ± 0.01[c]	1.7 ± 0.01[d]
12	2.7 ± 0.02[a]	3.0 ± 0.03[b]	5.9 ± 0.01[c]	2.5 ± 0.04[d]
15	3.0 ± 0.03[a]	3.4 ± 0.06[b]	7.4 ± 0.06[c]	2.9 ± 0.03[a]
18	3.6 ± 0.03[a]	4.6 ± 0.01[b]	8.2 ± 0.04[c]	3.5 ± 0.03[a]
21	4.2 ± 0.09[a]	4.9 ± 0.11[b]	8.6 ± 0.01[c]	4.0 ± 0.01[d]
24	4.8 ± 0.01[a]	5.4 ± 0.07[b]	9.5 ± 0.02[c]	4.7 ± 0.01[a]
27	5.3 ± 0.05[a]	6.6 ± 0.04[b]	9.9 ± 0.01[c]	5.2 ± 0.10[a]
30	6.0 ± 0.03[a]	7.0 ± 0.06[b]	11.0 ± 0.10[c]	5.8 ± 0.06[d]
33	6.7 ± 0.01[a]	7.2 ± 0.01[b]	11.6 ± 0.17[c]	6.4 ± 0.04[d]
36	7.0 ± 0.01[a]	7.9 ± 0.02[b]	13.1 ± 0.01[c]	6.5 ± 0.03[d]
39	7.7 ± 0.01[a]	8.2 ± 0.03[b]	13.2 ± 0.01[c]	-
42	7.7 ± 0.01[a]	8.2 ± 0.03[b]	13.3 ± 0.10[c]	-
45	-	-	14.0 ± 0.01	-

Different letters in the same row differ significantly ($p < 0.05$). The values represent the mean ± S.E. (n = 3). The growth of the plant is expressed in centimeters (Cm). The - denotes absence of growth.

Water absorption. respiration and germination were increased in a variety of seeds including winter wheat, maize and barley. The germination rate responded to the humic treatments but not to the percentage of viable seeds (Nardi *et al.,* 2016). There were positive responses from the treatment with humic products for tomatoes, cotton, grapes and sweet potatoes. Many authors have suggested mechanisms by which humic products could stimulate the growth of plants. The possibility of improving the availability and absorption of nutrients.

Metal chelation, increased water efficiency, improved permeability of the cell membrane, antioxidant and hormone-like effects and improved microbial metabolism were discussed (Arancon *et al.,* 2006).

The mechanism by which humic substances could promote the growth of plants is through the induction of the activity of H^+-ATPase, promoting the transport of secondary ions and the absorption of other nutrients. These channels facilitate the transport of nitrates through an electrochemical gradient of protons that induce the activity of this enzyme, the humic acid, when in contact with the cellular reticular cells (Sloboda *et al.*, 2017)

Humic substances promote plant growth through the acid growth of the root by proton increase, using the H^+-ATPase, pumping the apoplast, acidifying the cell wall, making it more flexible, which facilitates root elongation (Canellas and Olivares, 2014; Canellas *et al.*, 2015; Gomes De Melo *et al.,* 2016).

4. CONCLUSIONS

Humic acids stimulate growth in tomato (*Solanum lycopersicum*) and melon (*Cucumis melo*) plants. Considerable changes occur in the morphology of the plant. In the melon plant, growth is well known when the concentration of humic acid is increased above 0.1%, which did not happen with the tomato plants due to the lower increase at low concentrations, although higher concentrations of humic acid (>0.1%) accentuated the growth curve when compared against the control. This type of research can assist in the understanding of how humic substances influence plant biology and aid in the development of new technologies to increase the growth of plants based on humic material.

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